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# Interactions between uncoupling protein 2 gene polymorphisms, obesity and alcohol intake on liver function: a large meta-analysed population-based study

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<sup>\*</sup>(The details of the LifeLines Cohort Study is presented in the Acknowledgements section)

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## Abstract

**Background and objective:** Given the role of uncoupling protein 2 (UCP2) in the accumulation of fat in the hepatocytes and in the enhancement of protective mechanisms in acute ethanol intake, we hypothesised that *UCP2* polymorphisms are likely to cause liver disease through their interactions with obesity and alcohol intake. To test this hypothesis, we investigated the interaction between tagging polymorphisms in the *UCP2* gene (rs2306819, rs599277 and rs659366), alcohol intake and obesity traits such as BMI and waist circumference (WC) on alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) in a large meta-analysis of data sets from three populations ( $n=20\,242$ ).

**Design and methods:** The study populations included the Northern Finland Birth Cohort 1966 ( $n=4996$ ), Netherlands Study of Depression and Anxiety ( $n=1883$ ) and LifeLines Cohort Study ( $n=13\,363$ ). Interactions between the polymorphisms and obesity and alcohol intake on dichotomised ALT and GGT levels were assessed using logistic regression and the likelihood ratio test.

**Results:** In the meta-analysis of the three cohorts, none of the three *UCP2* polymorphisms were associated with GGT or ALT levels. There was no evidence for interaction between the polymorphisms and alcohol intake on GGT and ALT levels. In contrast, the association of WC and BMI with GGT levels varied by rs659366 genotype ( $P_{\text{interaction}}=0.03$  and  $0.007$ , respectively; adjusted for age, gender, high alcohol intake, diabetes, hypertension and serum lipid concentrations).

**Conclusion:** In conclusion, our findings in 20 242 individuals suggest that *UCP2* gene polymorphisms may cause liver dysfunction through the interaction with body fat rather than alcohol intake.

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## Introduction

Uncoupling protein 2 (*UCP2*) is a heat-generating mitochondrial inner-membrane carrier protein that is widely expressed in tissues including adipocytes (1) and liver micro-lobules (2). Although liver is the major site of *UCP2* expression in the rodent foetus, the levels decline soon after birth (3). However, under pathologic conditions such as during inflammation (4) or steatosis, it increases (5); *UCP2* gene expression has also been shown to occur in the hepatocytes of both normal adult rodents and healthy human adults, in the central area of the lobules (2). The role of *UCP2* in obesity and liver disease is substantiated by the evidence that *UCP2* enhanced fat accumulation in the liver, increased insulin resistance (6) and stimulated protective peripheral and central neural mechanisms in acute ethanol intake (7).

Polymorphisms within the *UCP2* gene have been shown to be associated with various metabolic traits such as obesity (8, 9) and type 2 diabetes (10, 11). Although the effects of these polymorphisms on *UCP2* function are still unclear, it could be hypothesised that the loss-of-function polymorphisms are likely to contribute to type 2 diabetes as a result of lower hepatic glucose uptake (12). This is due to reduced glucose reabsorption in the central areas of liver lobules, where this normally occurs (13) – a suggestion supported by the report of reduced type 2 diabetes risk with one *UCP2* polymorphism (14). There could also be an increase in the risk of metabolic acidosis due to the switch of ammonia detoxification from glutamine synthesis to ureogenesis (15). The gain-of-function polymorphisms in the *UCP2* gene could cause damage of centrilobular cells due to repeated episodes of undue hyperthermia, which is likely to occur during ethanol ingestion but not in non-drinkers (16).

Based on the hypothesis that *UCP2* gene polymorphisms can act as possible modulators of increases in liver enzymes through either fat accumulation or alcohol intake, we tested for the interaction effects between tagging single-nucleotide polymorphisms (tagSNPs) in the *UCP2* gene and alcohol intake and obesity traits on alanine aminotransferase (ALT) and gamma glutamyl

transferase (GGT), commonly used indicators of liver function, in a large meta-analysis of data sets from three populations (total *n* up to 20 242).

## Subjects and methods

Study participants from three studies – namely, the Northern Finland Birth Cohort 1966 (NFBC 1966; *n* up to 4996), Netherlands Study of Depression and Anxiety (NESDA; *n* up to 1883) and LifeLines Cohort Study (*n* up to 13 363) – were included in the meta-analysis.

### Data collection

NFBC 1966 was originally designed to study factors affecting pre-term birth, low birth weight and subsequent morbidity and mortality (17). Mothers living in the two Northern-most provinces of Finland were invited to participate if they had expected delivery dates during 1966. A total of 12 058 live births were included in the study. At age 31, all individuals still living in the Helsinki area or Northern Finland were asked to participate in a detailed biological and medical examination (*n*=6007) as well as a questionnaire. The sample size available for the present study was 5292 with data on genetic and phenotypic information. Written informed consent was obtained from all of the participants and the Ethics Committee of the Faculty of Medicine at the University of Oulu approved the study.

**Netherlands Study of Depression and Anxiety** ► NESDA is a multicentre study (18) designed to examine the long-term course and consequences of depressive and anxiety disorders (<http://www.nesda.nl>). NESDA included both individuals with depressive and/or anxiety disorders and controls without psychiatric conditions. Inclusion criteria were age 18–65 years and self-reported western European ancestry; exclusion criteria were not being fluent in Dutch and having a primary diagnosis of another psychiatric condition (psychotic disorder, obsessive compulsive

disorder, bipolar disorder or severe substance use disorder). The sample size available for the present study was 1925 with data on genetic and phenotypic information. Written informed consent was obtained from all the participants and the study was approved centrally by the Ethical Review Board of the VU University Medical Center and subsequently by local review boards of each participating centre.

**LifeLines Cohort Study** ► LifeLines is a multidisciplinary prospective population-based cohort study examining, in a unique three-generation design, the health and health-related behaviours of 167 729 persons living in the northeast region of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors that contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. The sample size available for the present study was 13 363 with data on genetic and phenotypic information. The LifeLines study has been approved by the review board of the University Medical Center, Groningen, and adheres to the principles expressed in the Declaration of Helsinki. All study participants provided written informed consent.

### Measurement of clinical parameters and variables

**Northern Finland Birth Cohort 1966** ► Anthropometric measures, including height, weight and waist circumference (WC), were measured without shoes and in underwear by a trained nurse. BMI was calculated as weight (kg)/height (m)<sup>2</sup>. The usage of alcohol was assessed by questionnaire (19). The alcohol variable was measured in g/day, calculated by the product of the frequency of how often alcoholic drinks are taken, which is converted to the proportion of days when drinking per year, the number of glasses taken when drinking and 12 (the average amount of grams of alcohol in a glass). ALT and GGT levels were measured by the Roche/Hitachi Modular Analyzer.

**Netherlands Study of Depression and Anxiety** ► Individuals who used antidepressants (not serotonin-specific reuptake inhibitors) were excluded from the analyses. Height, weight and WC were measured during a medical examination. BMI was calculated as weight (kg)/height (m)<sup>2</sup>. Data on alcohol use were collected using the Alcohol Use Disorder Identification Test questionnaire. The alcohol variable was measured in g/day, calculated by the product of the frequency of how often alcoholic drinks are

taken, which is converted to the proportion of days when drinking per year, the number of glasses taken when drinking and 12 (the average amount of grams of alcohol in a glass). Liver enzyme levels were determined with Roche Diagnostic assays.

**LifeLines Cohort Study** ► Height, weight and WC were measured during the nurse visit without shoes and in light clothing. BMI was calculated as weight (kg)/height (m)<sup>2</sup>. The alcohol variable was measured in number of glasses per month and subsequently converted to grams of alcohol per day using an average of 10 g alcohol/glass (10 g a day was based on the Dutch estimations of a glass per day). GGT and ALT plasma levels were determined by routine clinical chemistry at the UMCG (Groningen, The Netherlands) using the Roche/Hitachi Modular System (Roche).

### TagSNP selection

TagSNPs in the *UCP2* gene were selected using genotype data from the International HapMap collected in individuals of Northern and Western European ancestry (CEU; HapMap data release 24/phase II Nov 08, on NCBI B36 assembly, dbSNP b126). The Haploview Software V3.3 (<http://www.broadinstitute.org/haploview/haploview-downloads>) was used to assess the linkage disequilibrium structure between SNPs (20). Tagger Software (Cambridge, MA, USA) was used to select tagSNPs with the 'pairwise tagging only' option and an  $r^2$  threshold of  $>0.8$  ( $\pm 10$  kb upstream and downstream of the genes). In the tagSNP selection, we forced inclusion of the functional SNP previously studied (rs659366, -866 G/A promoter polymorphism) before running Tagger. At the time of the present study, we selected three tagSNPs – namely rs2306819, rs599277 and rs659366 – representing all of the common genetic variations within the *UCP2* gene according to the HapMap data release 24/phase II Nov 08, on NCBI B36 assembly, dbSNP b126. However, we capture only 75% of the *UCP2* gene variations according to the current HapMap data release 27/phases II + III, Feb 09, on NCBI B36 assembly, dbSNP b126.

### Genotyping

**Northern Finland Birth Cohort 1966** ► For NFBC, genomic DNA was extracted from whole blood using standard methods. All DNA samples for the Illumina Infinium 370cnvDuo array were prepared for genotyping by the Broad Institute Biological Sample Repository (BSP).

All individuals in the study were genotyped with call rates >95%. SNPs were excluded from analysis if the call rate in the final sample was <95%, if the *P* value from a test of Hardy–Weinberg equilibrium (HWE) was <0.0001, or if the minor allele frequency (MAF) was <1%.

**Netherlands Study of Depression and Anxiety** ► Individual genotyping was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays as part of the Genetic Association Information Network (GAIN) (21). Samples were excluded for failing quality control (QC) due to uncertain linkage between genotype and phenotype records, evidence of contamination, missing genotype data, high genome-wide homozygosity (~75%), first- or second-degree relationships or non-Caucasian ancestry. SNPs were required not to have any of the following features: gross mapping problem, greater than one genotype disagreements in 40 duplicated samples, greater than one Mendelian inheritance errors in complete trio samples, MAF <0.01 or >0.05 missing genotypes. A Hardy–Weinberg filter was not used. A total of 435 291 SNPs met these criteria and were included in the final analysis data set.

**LifeLines Cohort Study** ► Genotyping for Lifelines was performed on the Illumina CytoSNP12 v2 chip. Samples were excluded based on call rates below 0.95, gender mismatch, duplicate discordance and genetic similarity. Population stratification was assessed by principal component analysis over the sample correlation matrix, based on 16 842 independent SNPs. Samples were excluded when for they diverged from the mean with at least three s.d.s (*Z*-score >3) for the first five principal components. SNPs were excluded with a MAF of <0.01, call rate <0.95 or deviation from HWE ( $P < 1 \times 10^{-5}$ ). Genome wide genotype imputation was performed using Beagle v. 3.1.0, using the NCBI build 36 of phase II HapMap CEU data (release 22) as a reference panel.

### Statistical analysis

All of the SNPs were coded additively and with the effect allele as the minor allele. The metabolic measures GGT and ALT were highly skewed and were therefore dichotomised as normal or high using the population-based reference values (22). Individuals were considered to have high levels of ALT if their ALT value was >50 or 38 for men and women respectively. For GGT, the cut-off for high levels was 58 for men and 42 for women.

Logistic regression models were used to evaluate the interaction between the polymorphisms and BMI, WC and alcohol intake on the dichotomised GGT and ALT levels. In all three cohorts, logistic regression models were adjusted for age, gender, high alcohol intake (defined as the top 10% of the distribution for each sex), obesity (BMI <30 vs  $\geq 30$  kg/m<sup>2</sup>), diabetes, hypertension and lipid medication or hyperlipidaemia. In the presence of heterogeneity of effects between the studies, we used a random-effects meta-analysis; otherwise, we tested using fixed-effects inverse variance meta-analysis. All analyses were carried out using STATA (College Station, TX, USA), version 12. A *P* value of <0.05 was considered to be statistically significant.

### Results

As shown in Table 1, in the NFBC 1966 cohort, men had significantly higher BMI ( $P < 0.001$ ) and WC ( $P < 0.001$ ) and a significantly greater proportion of high GGT levels ( $P < 0.001$ ). No significant differences were observed between men and women for proportions of alcohol consumption ( $P = 0.96$ ) and high ALT levels ( $P = 0.94$ ). Similar findings were observed in NESDA and LifeLines, except for proportion of high ALT levels, which were significantly higher in men in both cohorts.

### Main effects

The genotype and allele frequencies of the three *UCP2* SNPs are presented in Table 2 and the three SNPs were in HWE ( $P > 0.05$ ). None of the three polymorphisms showed an association with ALT and GGT levels in the meta-analysis of the three cohorts (Table 3).

### Interaction of the *UCP2* polymorphisms with alcohol intake and obesity traits on ALT and GGT levels

Of the three *UCP2* polymorphisms, only SNP rs2306819 showed evidence for an interaction with alcohol intake on GGT levels in the NFBC 1966 ( $P_{\text{interaction}} = 0.02$ ). This interaction was, however, not observed in NESDA, LifeLines or in the meta-analysis of the three cohorts ( $P_{\text{interaction}} > 0.53$ , for all interactions). None of the other interactions with high alcohol intake on GGT levels were significant in the meta-analysis of the three cohorts ( $P_{\text{interaction}} > 0.54$ , for all; Table 4). There was also no evidence for interaction between the SNPs and alcohol intake on ALT levels in the meta-analysis ( $P_{\text{interaction}} > 0.26$ , for all).



Clinical Study	K S Vimalaswaran and others	UCP2 gene variants, obesity and liver function	173:6	867
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**Table 1** Clinical and biochemical characteristics of the study participants. Values represented are means  $\pm$  s.d.

Clinical and biochemical parameters	NFBC 1966		NESDA		LifeLines	
	Men (n=2535)	Women (n=2757)	Men (n=622)	Women (n=1303)	Men (n=5579)	Women (n=7784)
Age (years)	31	31	43.9 $\pm$ 12.0	40.9 $\pm$ 12.7	49.0 $\pm$ 11.7	48.5 $\pm$ 11.3
BMI (kg/m <sup>2</sup> )	25.2 $\pm$ 3.6	24.2 $\pm$ 4.7	26.3 $\pm$ 4.3	25.3 $\pm$ 5.3	26.7 $\pm$ 3.6	26.1 $\pm$ 4.7
WC (cm)	88.9 $\pm$ 9.9	78.9 $\pm$ 12.1	96.3 $\pm$ 13.2	85.4 $\pm$ 13.7	96.6 $\pm$ 10.5	88.5 $\pm$ 12.2
Obese (%)	8.3	9.5	17.9	16.5	15.4	17.3
Alcohol (g/day)	13.9 $\pm$ 20.6	4.9 $\pm$ 8.2	17.5 $\pm$ 22.1	9.2 $\pm$ 13.2	13.9 $\pm$ 12.8	4.1 $\pm$ 6.8
GGT (U/l) <sup>a</sup>	20 (14, 32)	11 (8, 16)	26 (17.8, 39)	15 (11, 22)	27 (20, 39)	17 (13, 23)
High GGT levels (%)	8.6	2.7	12.8	5.8	10.4	6.26
ALT (U/l) <sup>a</sup>	6 (2.5, 10)	5.5 (2.5, 9)	26.3 (19, 37)	16.9 (13, 22)	26 (20, 35)	17 (13, 22)
High ALT levels (%)	8.6	8.7	11.3	5.3	8.77	4.12

<sup>a</sup>For GGT and ALT levels, values presented are medians (interquartile range).

In contrast, in the analyses on obesity-related traits in the NFBC 1966, all of the three *UCP2* polymorphisms showed an interaction with BMI ( $P_{\text{interaction}} < 0.04$ ) and WC ( $P_{\text{interaction}} < 0.004$ ) on GGT levels, while two polymorphisms (rs599277 and rs659366) also suggested an interaction with obesity (as a binary trait) on GGT ( $P_{\text{interaction}} < 0.03$ ) (Table 5). The meta-analysis of the three cohorts confirmed the interactions between the SNP rs659366 and WC and BMI on GGT levels (WC,  $P_{\text{interaction}} = 0.03$  and BMI,  $P_{\text{interaction}} = 0.007$ ), while for the other two polymorphisms (rs599277 and rs2306819), evidence for interaction was not consistent; the SNP rs2306819 showed an interaction with WC ( $P_{\text{interaction}} = 0.04$ ) but not BMI ( $P_{\text{interaction}} = 0.08$ ) and obesity ( $P_{\text{interaction}} = 0.22$ ), and SNP rs599277 showed an interaction with BMI ( $P_{\text{interaction}} = 0.01$ ) and obesity ( $P_{\text{interaction}} = 0.03$ ) but not WC

( $P_{\text{interaction}} = 0.09$ ) (Table 5). None of the interactions between the three SNPs with obesity traits on ALT levels were statistically significant (data not shown).

## Discussion

The important finding of our study is that the effect of *UCP2* gene polymorphisms on liver function (as measured by GGT and ALT levels) is likely to be modified by levels of body fat rather than alcohol intake. Our meta-analysis in 20 242 individuals suggested that the functional *UCP2* gene polymorphism, rs65966, interacts with both WC (measure of central body fat distribution) and BMI (measure of body fat levels) on its influence on GGT levels. This finding is in line with the role of *UCP2* in the accumulation of fat in the hepatocytes (6), which is likely

**Table 2** The genotype and allele frequencies of the three *UCP2* gene polymorphisms in the three cohorts stratified by gender.

UCP2 polymorphisms	NFBC 1966		NESDA <sup>a</sup>		LifeLines	
	Men	Women	Men	Women	Men	Women
rs2306819						
CC	1232	1329	276.11	584.61	2188	3056
CT	1051	1132	296.02	569.78	2801	3862
TT	236	272	49.62	148.17	590	866
MAF (%)	30	31	32	33	36	36
rs599277						
GG	640	700	200.41	396.30	1715	2415
AG	1202	1332	317.42	638.30	2806	3827
AA	621	668	103.77	267.48	1058	1542
MAF (%)	50	49	42	45	44	44
rs659366						
CC	944	1024	248.99	514.22	2179	3022
CT	1201	1309	295.03	616.22	2616	3610
TT	395	432	77.65	171.85	784	1152
MAF (%)	39	39	36	37	37	38

MAF, minor allele frequency.

<sup>a</sup>Imputed genotypes.

Clinical Study	K S Vimalaswaran and others	UCP2 gene variants, obesity and liver function	173:6	868
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**Table 3** Association of the *UCP2* gene polymorphisms with ALT and GGT levels.

Study cohorts	rs2306819		rs599277		rs659366	
	$\beta \pm \text{s.e.m.}$	<i>P</i> value*	$\beta \pm \text{s.e.m.}$	<i>P</i> value*	$\beta \pm \text{s.e.m.}$	<i>P</i> value*
ALT levels						
NFBC 1966	$-0.03 \pm 0.09$	0.74	$-0.11 \pm 0.08$	0.17	$-0.09 \pm 0.08$	0.30
NESDA	$0.09 \pm 0.15$	0.54	$0.04 \pm 0.14$	0.79	$-0.19 \pm 0.15$	0.19
LifeLines	$-0.03 \pm 0.06$	0.65	$0.01 \pm 0.05$	0.80	$0.03 \pm 0.05$	0.62
Meta-analysis	$0.05 \pm 0.68$	0.68	$0.04 \pm 0.63$	0.63	$0.04 \pm 0.57$	0.57
GGT levels						
NFBC 1966	$-0.14 \pm 0.11$	0.21	$-0.002 \pm 0.09$	0.98	$0.04 \pm 0.10$	0.67
NESDA	$0.01 \pm 0.15$	0.93	$-0.10 \pm 0.14$	0.46	$-0.17 \pm 0.15$	0.23
LifeLines	$0.04 \pm 0.05$	0.42	$0.06 \pm 0.05$	0.24	$0.07 \pm 0.05$	0.14
Meta-analysis	$0.008 \pm 0.04$	0.85	$0.03 \pm 0.04$	0.44	$0.05 \pm 0.04$	0.27

\**P* values adjusted for age, gender, high alcohol intake, obesity, diabetes, hypertension and lipid medication/hyperlipidaemia.

to result in an elevation of GGT levels due to resultant liver dysfunction.

Given the role of *UCP2* in the control of energy metabolism (23), polymorphisms in the *UCP2* gene have been examined for their associations with obesity and its related traits. The three commonly studied polymorphisms in the *UCP2* gene are –866G/A polymorphism in the promoter region, the Ala55Val polymorphism in the exon 4 and the 45 bp insertion/deletion polymorphism in the 3'-UTR. These polymorphisms have been shown to be associated with various metabolic traits such as obesity (24), body fat distribution (25), resting energy expenditure (26), type 2 diabetes (10) and insulin resistance (9, 27) in various populations from different ethnic backgrounds (24). However, there were also studies that failed to show an association between these polymorphisms and metabolic traits (8, 28, 29). These inconsistencies could be attributed to the small sample size, incomplete coverage of the *UCP2* gene variations or potential population-specific influences on metabolic traits (29). Hence, in our study we

have performed a meta-analysis of data from three large European cohorts (*n* up to 20 242) and used the tagSNP approach to capture all common genetic variations within the *UCP2* gene. Even though the study protocol used in the three cohorts were different and the participants were from different geographical regions and age groups, the three cohorts were of European ethnicity and the analyses were adjusted for age, gender, alcohol consumption, obesity, diabetes, hypertension and lipid medication. However, we cannot completely rule out the effects of other diseases (such as mental health-related outcomes in NESDA) on our analysis.

Independent of the associations with obesity and diabetes, *UCP2* has also been implicated in non-alcoholic fatty liver disease (NAFLD) (30). *UCP2* gene expression has been shown to be increased in the liver of rats with NAFLD (31); however, the real effect of *UCP2* on NAFLD is still unclear. Several mechanisms have been hypothesised (6, 30), and the accumulating evidence supports the role of mitochondrial dysfunction in NAFLD, in which *UCP2*

**Table 4** Interaction between *UCP2* gene polymorphisms and high alcohol intake on ALT and GGT levels.

Study cohorts	rs2306819	rs599277	rs659366
$\beta$ coefficients $\pm$ s.e.m. ( <i>P</i> values*) for interaction on ALT levels			
NFBC 1966	$-0.14 \pm 0.27$ (0.61)	$0.16 \pm 0.23$ (0.50)	$0.11 \pm 0.24$ (0.64)
NESDA	$0.05 \pm 0.38$ (0.89)	$0.35 \pm 0.37$ (0.35)	$0.25 \pm 0.39$ (0.52)
LifeLines	$0.14 \pm 0.16$ (0.37)	$0.16 \pm 0.15$ (0.29)	$0.13 \pm 0.15$ (0.40)
Meta-analysis	$0.07 \pm 0.13$ (0.59)	$0.18 \pm 0.12$ (0.89)	$0.14 \pm 0.12$ (0.26)
$\beta$ coefficients $\pm$ s.e.m. ( <i>P</i> values*) for interaction on GGT levels			
NFBC 1966	$0.58 \pm 0.25$ (0.02)	$0.39 \pm 0.23$ (0.09)	$0.42 \pm 0.23$ (0.07)
NESDA	$-0.13 \pm 0.35$ (0.70)	$-0.09 \pm 0.33$ (0.77)	$-0.24 \pm 0.35$ (0.49)
LifeLines	$-0.03 \pm 0.13$ (0.79)	$-0.005 \pm 0.12$ (0.97)	$-0.06 \pm 0.12$ (0.62)
Meta-analysis	$0.07 \pm 0.11$ (0.53)	$0.06 \pm 0.09$ (0.54)	$0.02 \pm 0.10$ (0.85)

\**P* values adjusted for age, gender, obesity, diabetes, hypertension and lipids.

Clinical Study	K S Vimalaswaran and others	UCP2 gene variants, obesity and liver function	173:6	869
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**Table 5** Interaction between *UCP2* gene polymorphisms and obesity, BMI and WC on GGT levels.

Study cohorts	rs2306819	rs599277	rs659366
$\beta$ coefficients $\pm$ s.e.m. ( <i>P</i> values*) for interaction between polymorphisms and obesity on GGT levels			
NFBC 1966	0.49 $\pm$ 0.27 (0.07)	0.55 $\pm$ 0.26 ( <b>0.03</b> )	0.63 $\pm$ 0.28 ( <b>0.02</b> )
NESDA	0.26 $\pm$ 0.32 (0.41)	0.64 $\pm$ 0.32 ( <b>0.04</b> )	0.83 $\pm$ 0.33 ( <b>0.01</b> )
LifeLines	0.04 $\pm$ 0.11 (0.71)	0.10 $\pm$ 0.10 (0.33)	0.12 $\pm$ 0.11 (0.27)
Meta-analysis	0.12 $\pm$ 0.09 (0.22)	0.20 $\pm$ 0.09 ( <b>0.03</b> )	0.24 $\pm$ 0.09 ( <b>0.01</b> )
			( <i>P</i> =0.06 if using RE meta-analysis, p-het=0.04)
$\beta$ coefficients $\pm$ s.e.m. ( <i>P</i> values*) for interaction between polymorphisms and BMI on GGT levels			
NFBC 1966	0.04 $\pm$ 0.02 ( <b>0.04</b> )	0.05 $\pm$ 0.02 ( <b>0.01</b> )	0.05 $\pm$ 0.02 ( <b>0.02</b> )
NESDA	0.02 $\pm$ 0.03 (0.40)	0.04 $\pm$ 0.03 (0.10)	0.02 $\pm$ 0.03 (0.46)
LifeLines	0.007 $\pm$ 0.01 (0.47)	0.01 $\pm$ 0.009 (0.22)	0.02 $\pm$ 0.009 (0.07)
Meta-analysis	0.02 $\pm$ 0.009 (0.08)	0.02 $\pm$ 0.008 ( <b>0.01</b> )	0.02 $\pm$ 0.008 ( <b>0.007</b> )
$\beta$ coefficients $\pm$ s.e.m. ( <i>P</i> values*) for interaction between polymorphisms and WC on GGT levels			
NFBC 1966	0.02 $\pm$ 0.008 ( <b>0.005</b> )	0.03 $\pm$ 0.008 ( <b>0.0003</b> )	0.03 $\pm$ 0.008 ( <b>0.001</b> )
NESDA	0.001 $\pm$ 0.01 (0.94)	0.009 $\pm$ 0.01 (0.36)	0.01 $\pm$ 0.01 (0.26)
LifeLines	0.004 $\pm$ 0.00 (0.28)	0.005 $\pm$ 0.004 (0.19)	0.007 $\pm$ 0.004 ( <b>0.05</b> )
Meta-analysis	0.007 $\pm$ 0.003 ( <b>0.04</b> )	0.009 $\pm$ 0.003 ( <b>0.006</b> )	0.01 $\pm$ 0.006 ( <b>0.001</b> )
		( <i>P</i> =0.09 with RE meta-analysis, p-het=0.03)	( <i>P</i> =0.03 with RE meta-analysis, p-het=0.03)

RE, random-effects; p-het, *P* value for heterogeneity between the studies. \**P* values adjusted for age, gender, high alcohol intake, diabetes, hypertension and lipids.

plays a vital role through its uncoupling of oxidative phosphorylation (32). Further to the role of *UCP2* in NAFLD, *UCP2* also has shown to stimulate protective peripheral and central neural mechanisms in acute ethanol intake (7). Hence, we aimed to investigate the hypothesis (2) that alcohol intake could have an adverse effect on liver function in individuals with gain of function polymorphisms of the *UCP2* gene that is not seen in non-drinkers. One of the reasons for alcohol intake not being an effect modifier in the link between *UCP2* polymorphisms and GGT/ALT levels in this large-scale meta-analysis could be because of the fact that the effect of drugs on the observed association was not excluded.

Even though the interaction between the *UCP2* gene polymorphism, rs659366, and WC and BMI on GGT levels was not significant in all the cohorts, there was a consistent interaction of rs659366 with WC and BMI on GGT levels in the meta-analysis of the three cohorts. Findings from previous studies (25, 33) have also implicated the role of *UCP2* gene polymorphism, rs659366, in WC and body fat composition, and hence, our results are suggestive of the role of body fat as a possible effect modifier of the link between *UCP2* and liver dysfunction.

The interaction between *UCP2* and WC was observed only when examining GGT levels as an outcome. Although both ALT and GGT levels have been used as indicators of liver dysfunction, GGT levels have been shown to be better than ALT as predictors of metabolic

traits such as diabetes (34). Also, some studies have demonstrated a stronger association between GGT and diabetes than between ALT and diabetes (35, 36). Furthermore, GGT levels have been considered to have the potential to be included in algorithms for metabolic syndrome, because the predictive value in the diagnosis of metabolic syndrome is higher for GGT as compared to ALT (37). Hence, it is possible that the GGT levels, rather than ALT, might be a marker of a mechanism that is responsible for interaction between the *UCP2* polymorphism and body fat in our study.

Previous studies have functionally characterized the role of the *UCP2* gene promoter polymorphism –866 G/A (rs659366) in obesity and diabetes (38, 39). The WT G allele of the polymorphism has been shown to be associated with enhanced adipose tissue mRNA expression *in vivo*, reduced transcriptional activity *in vitro* and increased risk of obesity (39). Also, the same polymorphism has been shown to affect the preferential binding of PAX6 (a pancreatic transcription factor involved in pancreatic  $\beta$ -cell development and function) to the *UCP2* gene promoter (38). In line with the functionality of this polymorphism, our finding raises the question of whether this polymorphism interacts with body fat in altering GGT levels through a pathway independent of obesity and diabetes. Hence, mechanistic studies are required to understand the role of this functional polymorphism in relation to body fat and liver function.



In summary, our study, in up to 20 242 individuals, shows evidence for an interaction between *UCP2* gene polymorphism, rs659366, and WC and BMI on GGT levels, suggesting that *UCP2* gene polymorphisms may cause liver dysfunction through the interaction with body fat rather than alcohol intake. Further large studies are required to confirm this finding and should focus on considering the effect of drugs when testing for the interaction with alcohol intake.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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#### Author contribution statement

K S Vimalaswaran, R D Cohen, B J Boucher and E Hyppönen conceived and designed the study and drafted the manuscript; A Cavadino, N Verweij,

I M Leach, J Auvinen, P Elliott, B W Penninx, M-R Järvelin and P v d Harst contributed to the study design; A Cavadino performed the statistical analysis; I M Nolte, I M Leach, J Veijola, H Snieder and B J Boucher provided a critical review of the manuscript. All authors read and approved the final manuscript.

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